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Toxicological and Physiological Responses of the Fish, 'Leiostomus xanthurus' Exposed to Chlorine Produced Oxidants

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16. ABSTRACT

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Opecular ventilation rates in exposed spot were much higher than in control fish, but returned to rates only slightly above those of controls during the latter portion of the 3,880 minute exposure of the two sublethal CPO concentrations. Opercular rates at the three acutely toxic CPO concentrations remained much higher than control rates until the exposed fish died.

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Toxicological and Physiological Responses of the Fish, *Leiostomus xanthurus*, Exposed to Chlorine Produced Oxidants[1]

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ABSTRACT: The sublethal and lethal effects of chlorine produced oxidants (CPO) on juveniles of the estuarine teleost, *Leiostomus xanthurus*, were investigated in flowing water tests conducted at 30 ± 1 °C and 26 to 31‰ salinity. Short-term LT50 tests were conducted at two nominal concentrations of NaOCl, 1.0 and 1.4 mg/l (respective measured CPO concentrations 0.09 and 0.12 mg/l) which were sublethal in 2,880 minute exposures; and three nominal concentrations, 1.6, 1.8 and 3.2 mg/l NaOCl (respective measured CPO concentrations 0.13, 0.20 and 0.37 mg/l) which were acutely toxic.

Opercular ventilation rates in exposed spot were much higher than in control fish, but returned to rates only slightly above those of controls during the latter portion of the 2,880 minute exposure to the two sublethal CPO concentrations. Opercular rates at the three acutely toxic CPO concentrations remained much higher than control rates until the exposed fish died.

Blood pH after 2,880 minutes of exposure to the sublethal concentrations of CPO; or at the respective estimated LT50 for lethal concentrations, showed significant decreases ($\bar{X}$ as low as 6.84) compared to controls ($\bar{X}$ 7.35). No significant changes in the % methemoglobin were observed.

Oxygen uptake by spot was depressed at all of the measured concentrations of CPO tested. Histopathological examination showed that gill respiratory epithelial tissues sloughed away from the underlying pillar cells. Complete denudation of circulatory tissues and hemangiectic secondary lamellae were observed in gill tissues from fish exposed to the highest CPO concentration of 0.37 mg/l.

Introduction

Several studies have documented the toxicological, physiological and behavioral responses of freshwater and marine teleosts exposed to chlorinated secondary sewage treatment wastes and chlorinated water used for single-pass cooling in thermal electric generating plants (Merkens 1958; Tsai 1968, 1970; Zillich 1972; Alderson 1974; Meldrim, et al. 1974; Servizi and Martens 1974; Middaugh, et al. 1977a, b; 1978). In freshwater fishes, it has been shown that acutely toxic levels of chlorination result in specific responses prior to death. These re-

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sponses include production of copious quantities of mucus on the body surface and especially the gills (Dandy 1972), increased opercular ventilation and coughing rates (Bass and Heath 1977; Zeitoun 1977) and gill damage including hyperplasia, neucroses and sloughing of epithelial tissues (Servizi and Martens 1974; Cohen and Valenzuela 1977). In addition, Bass and Heath (1977) measured decreased blood pH and increased lactate levels, and a slight elevation of methemoglobin (approximately 2% above control values) in chlorination exposed rainbow trout, *Salmo gairdneri*, but indicated that the increase in methemoglobin probably was not physiologically significant. In contrast, Grothe and Eaton (1975) reported physiologically significant increases in methemoglobin (approximately 30% above control values) for fathead minnows, *Pimephales promelas*, exposed to acutely toxic concentrations of chloramines. Their tests suggested that decreased oxygen carrying capacity of the hemoglobin resulted in hypoxia and eventually death.

Similar responses have been demonstrated for estuarine and marine fishes exposed to acutely toxic levels of chlorine produced oxidants (CPO). Juvenile spot, *Leiostomus xanthurus*, showed apparent increases in opercular ventilation rates and accumulation of mucus along the posterior edge of the operculum during exposure to CPO (Middaugh, et al. 1977b); juvenile striped bass, *Morone saxatilis*, responded similarly. Histological examination indicated gill damage in both species (Middaugh, et al. 1977a, b).

While toxicological responses of freshwater and estuarine or marine fishes exposed to CPO appear to be analogous, little information is available on the physiology of marine fishes exposed to CPO. The present study measured toxicological and concurrent physiological responses of juvenile spot, *Leiostomus xanthurus*, exposed to sublethal and lethal levels of CPO.

Initially, median lethal time (LT50) tests were conducted at 5 measured CPO concentrations. These tests were repeated and spot monitored at specific time intervals for opercular ventilation rates, blood pH, % methemoglobin, whole animal respiration, and histopathological response.

Materials and Methods

Juvenile spot (4.6 to 9.0 cm standard length; 1.4 to 14.4 g wet weight) were seined from Leadenwah Creek, a tidal tributary of the North Edisto River estuary in South Carolina during June and July 1977. Water temperature during collections ranged from 27.5 to 30.0 °C, and the salinity from 29 to 30‰. Spot were maintained in the laboratory in 80 l rectangular fiberglass flow-through aquaria (60 cm L × 50 cm W × 25 cm D) which received 60 l/hour of seawater filtered through crushed oyster shell. Water temperature was maintained at 30 ± 1 °C with quartz immersion heaters during pretest acclimation and all subsequent experiments. The salinity of flowing seawater in the laboratory ranged from 26 to 31‰ and the pH from 7.8 to 8.0 units. Periodic measurement of dissolved oxygen in holding and treatment aquaria showed that concentrations were >90% of air saturation. Spot were held in the laboratory for at least 5 days before use in tests. They were fed grass shrimp, *Palaemonetes pugio*, each morning until 24 hours prior to experiments. A photoperiod of 14 hours light: 10 hours dark with a light intensity of 1600 lux, was provided by "cool white" fluorescent lamps suspended 1.7 m above acclimation and experimental aquaria.

Toxicity Tests

To determine the LT50 for specific measured CPO concentrations, 20 spot were placed in a 25 l fiberglass flow-through aquarium (56 cm L × 29 cm W × 15 cm D) which received 36 l/hour of temperature controlled (30 ± 1 °C) seawater. After 12 to 18 hours of acclimation to the treatment aquarium, chlorine (Fisher Analytical Reagent, 4 to 6% NaOCl) was introduced by syringe pump. A vertical-standing perforated PVC pipe served as a "mixing chamber" at the point of seawater and NaOCl introduction into the aquarium. A stand-pipe-drain was located at the opposite end of the treatment aquarium. A total of 5 measured CPO concentrations were used to determine LT50 responses. Each test was replicated at each CPO concentration.

Observations for death in the LT50 tests were made continuously for the three high-

est CPO concentrations and at 1 to 8 hour intervals for the two lowest concentrations. The criteria for death were cessation of opercular movement and failure to respond when touched with a glass rod. All tests were terminated after 2,800 minutes (48 hours) of exposure. An untreated control aquarium was maintained in each test. Control survival was 95% or more in all tests. Methods described by Litchfield (1949) and Litchfield and Wilcoxon (1949) were used to analyze the LT50 data.

After LT50 responses had been determined in replicate tests at each CPO concentration, the tests were repeated several times (using new fish in each test). During these tests, opercular ventilation rates were noted at specific time intervals. In addition, fish were sacrificed at the estimated LT50 (the average value for replicate tests was used), and measurements of blood pH and % methemoglobin were made. Whole animal respiration ($\dot{V}_{O_2}$) in ml $O_2 \cdot$ kg wet weight $\cdot$ hour^{-1} was also determined at specific time intervals after beginning CPO exposures.

Opercular Ventilation Rates

Our exposure procedures were as described in the preceding section. Experimental and control aquaria were shielded from outside disturbances. Visual counts of opercular ventilation rates were made for 8 fish exposed to each concentration of CPO and 8 control fish at intervals of 0 (prior to exposures), 15, 30, 60, 90, 120, 240, 280, 720, 1,440 and 2,880 minutes after beginning introduction of NaOCl to exposure aquaria.

Data were analyzed by the binomial expansion to determine if significant differences ($p \leq 0.05$ or 0.10) occurred between the means for control and experimental ventilation rates (Sokal and Rohlf 1969).

Blood pH and % Methemoglobin Measurements

Blood samples were obtained by severing the tail and rapidly collecting blood in heparinized capillary tubes. Spot were disturbed as little as possible prior to sampling. Blood samples from individual spot were sealed in capillary tubes with clay and placed on ice for subsequent pH determinations using a Radiometer Copenhagen Acid-Base Analyzer (PHM-71)[2] thermostated to 30 °C. Garey (1972), however, has determined that this sampling technique yields blood pH values much lower than when samples are obtained via indwelling catheters. Nonetheless, the methods employed in this study appear to be sensitive to relative changes in blood pH in juvenile fish too small for catheters.

The % blood methemoglobin level was measured by the method of Hegesh, et al. (1970). In many instances, blood samples from 2 or 3 spot were pooled to obtain a sample large enough for the methemoglobin analysis. The students t-test was used to determine if significant differences ($p \leq 0.05$) existed between the means for control and experimental spot for pH and methemoglobin measurements.

Oxygen Uptake

The rate of oxygen uptake ($\dot{V}_{O_2}$) in ml $O_2 \cdot$ kg wet weight $\cdot$ hour^{-1} was measured by monitoring oxygen depletion by spot held in a closed system. Each fish was placed in a 500 ml Erlenmeyer flask filled with filtered seawater (0.45 μm) immersed in a constant temperature (30 $\pm$ 0.5 °C) water bath. The flask was closed from the atmosphere with a self stirring oxygen electrode (Yellow Springs Instrument Co. Model 5920). Oxygen levels were monitored with an oxygen meter (YSI Model 54) and strip chart recorder. Each fish was allowed to remain in the flask for at least 20 minutes prior to $\dot{V}_{O_2}$ determinations. The flask was perfused with aerated seawater through vents until the oxygen levels returned to at least 90% of air saturation. The rate of oxygen depletion was then monitored for approximately 30 minutes.

Histology

Surviving spot were sacrificed for histological examination at the estimated LT50 for respective CPO exposure concentrations. For the two sublethal concentrations (0.09 and 0.12 mg/l CPO) and controls, individuals were sacrificed after 2,880 minutes in experimental aquaria. Spot were placed in Davidson's Fixative for 24 hours, gill

[2] Mention of trade names does not imply endorsement by the U.S. Environmental Protection Agency.

TABLE 1. Acute toxicity data for replicate tests with spot exposed to 5 nominal NaOCl concentrations. Measured mean ($\bar{X}$) chlorine produced oxidant (CPO) values and standard deviation (S) are given for each test. The time to 50% mortality (LT50) and 95% confidence intervals (95% C.I.); and the slope of the effect-time curve and 95% C.I. are given for each test.

Replicate Test	Nominal NaOCl Conc. mg/l	N	Measured CPO Conc. mg/l $\bar{X}$	Measured CPO Conc. mg/l S	LT50 (min)	95% C.I. (min)	Slope of Curve	95% C.I. of Curve
A	1.0	5	0.09	0.005	>2,880	—	—	—
B	1.0	5	0.09	0.007	>2,880	—	—	—
A	1.4	5	0.12	0.01	>2,880	—	—	—
B	1.4	5	0.12	0.01	>2,880	—	—	—
A	1.6	5	0.13	0.02	360	285–454	1.66	1.38–1.99
B	1.6	5	0.13	0.01	400	296–540	1.93	1.54–2.41
A	1.8	5	0.19	0.02	216	183–254	1.44	1.26–1.64
B	1.8	5	0.20	0.02	250	217–288	1.38	1.25–1.52
A	3.2	5	0.37	0.03	88	81–95	1.20	1.11–1.27
B	3.2	5	0.36	0.02	95	90–100	1.12	1.08–1.16

Control mortality <5% for all tests.

arches were excised, placed in 50% ethanol for 2 hours and finally transferred to 70% ethanol. Gill arches were dehydrated, infiltrated with paraffin, and paraffin-embedded for sagittal and parasagittal sectioning at 7 μm. Sections were strained with Harris hematoxylin and eosin and mounted for light microscopy.

Chemical Analysis

Chlorine produced oxidant (CPO) concentrations (free + combined chlorine and other chlorine produced oxidants) resulting from the addition of NaOCl (Fisher Analytical Reagent, minimum 4 to 6% NaOCl) to seawater were measured with a Wallace and Tiernan Amperometric titrator. Phenylarsine oxide and pH buffers were standard Wallace and Tiernan reagents. The 5% KI solution and NaOCl stock solutions were prepared in chlorine demand-free water. Stock solutions for chemical analysis and biological tests were stored in brown glass bottles at 4 °C prior to use. Chlorine produced oxidant determinations were conducted by procedures outlined in Standard Methods (APHA 1971). All samples were taken directly from the exposure aquaria and titrated to an end point within one minute. Agitation of samples was minimized to reduce potential error caused by flashing-off or photodecomposition of chlorine produced oxidants (EPA 1974).

The use of the term "chlorine produced oxidant" (CPO) in this paper to describe the active oxidants measured by the amperometric titration method supercedes previous designations such as total residual chlorine and total residual chlorination (TRC). Recent evidence has shown that chlorine added to seawater in the form of NaOCl, $Ca(OCl)_2$ or Cl_2 gas, reacts in a similar way to form several "chlorination" products including but not limited to hypochlorous acid, hypochlorite ion, hypobromous acid and hypobromite ion. Concentrations of each chlorine produced oxidant resulting from the addition of chlorine to seawater will vary according to physical and chemical parameters of the seawater (Dove 1970, Burton 1977; Johnson 1977; Sugam and Helz 1977; Macalady, et al. 1977).

Results

The measured CPO concentrations reported for each nominal NaOCl concentration indicated that approximately 10% of the NaOCl added at each nominal exposure concentration was present in the treatment aquaria as measurable chlorine produced oxidants (Table 1). There were no mortalities in juvenile spot, *Leiostomus xanthurus*, exposed to the two lowest (CPO) concentrations (0.09 and 0.12 mg/l) for 2,880 minutes but the three highest concentrations (0.13 to 0.37 mg/l) were acutely toxic (Table 1). The estimated LT50 values for the replicate test at each exposure concentration of CPO were similar, as were the estimated slopes for each toxicity curve.

Opercular ventilation rates for spot exposed to all 5 CPO concentrations showed

TABLE 2. Mean opercular ventilation rates for eight control and experimental spot exposed to 5 concentrations of CPO. The binomial expansion $(p + q)^n$ was used to test the hypothesis of no difference between control and experimental opercular ventilation rates. Probability ≤ 0.05 or 0.01 for columns of control and experimental values at respective measured CPO concentrations.

Exposure Time (Min)	Measured CPO Concentrations									
	0.09 mg/l x̄ beats/min		0.12 mg/l x̄ beats/min		0.13 mg/l x̄ beats/min		0.20 mg/l x̄ beats/min		0.37 mg/l x̄ beats/min	
	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl
0	66	57	66	68	64	59	88	102	82	85
15	65	96	65	99	68	129	70	163	72	189
30	67	114	67	155	65	146	63	209	74	217
60	64	93	64	121	62	128	60	197	77	154†
90	63	93	62	93	67	149	70	197	—	—
120	61	88	61	125	71	156	83	196	—	—
240	66	92	66	115	63	200	82	124*	—	—
480	72	100	71	110	65	162*	—	—	—	—
720	74	87	74	87	—	—	—	—	—	—
1,440	71	76	72	80	—	—	—	—	—	—
2,880	68	75*	78	78*	—	—	—	—	—	—

*$p \leq 0.05$; † $p \leq 0.01$; — No experimental (CPO exposed) survivors.

significant differences (increases) when compared to controls (Table 2). However, a return to opercular ventilation rates only slightly higher than those observed in the controls occurred after 720, 1,440 and 2,880 minutes in spot exposed to 0.09 mg/l and after 1,440 and 2,880 minutes in spot exposed to 0.12 mg/l CPO. In the three highest concentrations of CPO (0.13, 0.20 and 0.37 mg/l) a slight decrease in opercular rates was noted just prior to death (Table 2).

Blood pH decreased significantly in all of the CPO exposure concentrations except 0.09 mg/l, in comparison to the control values. The % methemoglobin in exposed spot was not different from controls (Table 3).

Oxygen uptake rates ($\dot{V}_{O_2}$) for CPO exposed spot were significantly different from control spot, with one exception (Table 4). At the sublethal CPO concentrations (0.09 and 0.12 mg/l) $\dot{V}_{O_2}$ was 56.6 and 55.6% of control $\dot{V}_{O_2}$ respectively, after 2,880 minutes exposure. Oxygen uptake by spot surviving 380 minutes exposure to 0.13 mg/l CPO, a lethal concentration, was reduced to 73.8% of the control value. Oxygen uptake in spot exposed to 0.20 mg/l CPO was 95% of control spot after 90 minutes and 65.9% after 180 minutes, the LT50. For the highest CPO concentration tested (0.37 mg/l), $\dot{V}_{O_2}$ was depressed to 65.8% of the control value after 30 minutes and 30.4% after 60 minutes. The LT50 at 0.37 mg/l CPO was approximately 90 minutes.

In assessing the histological effects of CPO, excised whole branchial arches were studied with particular attention given to lamellae of the branchiae. Control fish had

TABLE 3. Blood pH and % methemoglobin for control spot and those exposed to 5 CPO concentrations. Fish at 0.09 and 0.12 mg/l CPO sacrificed after 2,880 minutes exposure, others at the estimated LT50 for respective concentrations of CPO.

	Exposure Concentration of CPO (mg/l)											
	Control		0.09		0.12		0.13		0.20		0.37	
	pH	% Met.	pH	% Met.	pH	% Met.	pH	% Met.	pH	% Met.	pH	% Met.
x̄	7.35	0.29	7.37	0.47	7.27	0.30	7.13	0.31	6.84	0.44	7.01	0.32
Min.	7.06	0.00	7.14	0.00	7.05	0.12	7.01	0.00	6.63	0.18	6.84	0.00
Max.	7.53	0.86	7.53	1.53	7.47	0.57	7.26	0.96	6.98	0.91	7.22	0.75
N	21	9	15	10	16	8	14	10	16	13	11	13
$S_{\bar{x}}$	0.02	0.09	0.03	0.14	0.03	0.07	0.02	0.10	0.02	0.07	0.03	0.08
t	—	—	0.50	1.08	2.25*	0.09	6.70*	0.15	15.48*	1.35	8.54*	0.26

* $p \leq 0.05$.

TABLE 4. Oxygen uptake rates ($\dot{V}_{O_2}$) in ml·kg·h^{-1} for spot exposed to 5 CPO concentrations for the time intervals indicated.

	O_2 uptake ml·kg·h^{-1}							
Exposure Conc. CPO (mg/l)	Control	0.09	0.12	0.13	0.20		0.37	
Exposure Time (min)	000	2,880	2,880	380	90	180	30	60
$\bar{x}$	392.15	218.23	222.05	289.41	372.86	258.31	297.12	119.33
N	11	10	10	10	10	11	10	10
$S_{\bar{x}}$	28.22	12.33	8.10	25.15	25.30	24.78	34.35	14.54
t	—	5.46*	5.55*	2.70*	0.51	3.57*	2.15*	8.34*

* $p \leq 0.05$.

normal gills with the exception of slight focal edematous separations of respiratory epithelium from underlying circulatory tissue (pillar cells) (Fig. 1). These focal separations of tissues were not extensive and are probably a preparation artifact. Gills of fish exposed to concentrations of 0.09 and 0.12 mg/l CPO had extensive separation of lamellar respiratory epithelia and underlying circulatory tissues (Fig. 2). This condition was not focal but occurred throughout the branchiae. Fish exposed to CPO concentrations of 0.13, 0.20, and 0.37 mg/l CPO suffered extensive damage to the respiratory epithelium of their lamellae (Figs. 3, 4, 5 and 6). Exposure to the intermediate concentrations resulted in separation and abnormal fusion of respiratory epithelia of adjacent lamellae (Figs. 4 and 5) whereas exposure to a concentration of 0.37 mg/l CPO resulted in complete denudation of the circulatory tissues of the lamellae (Fig. 6).

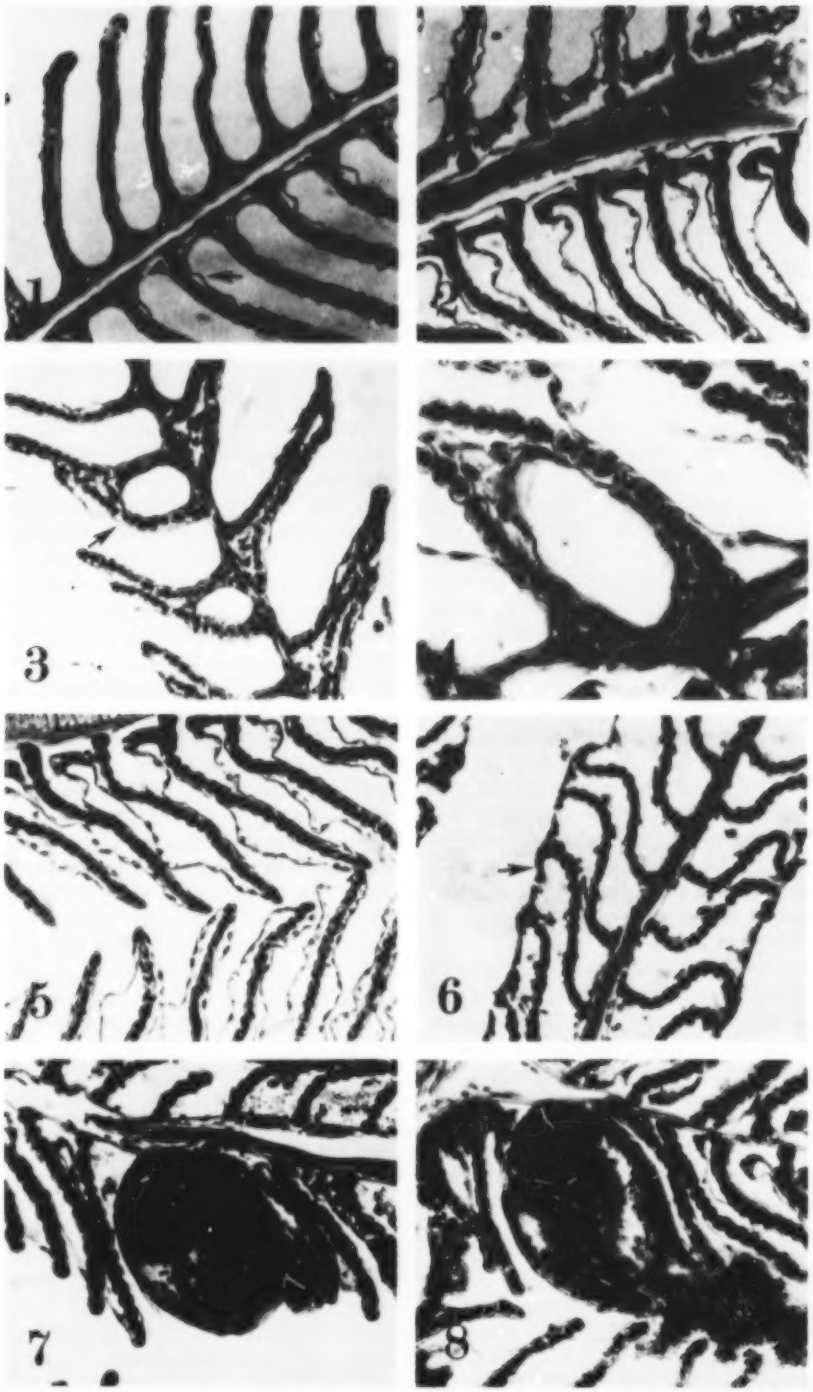
Another finding in CPO exposed fish was hemangiectasis of blood vessels of lamellae proximal to the gill arch (Fig. 7). This condition resulted in large contained masses of red blood cells. Any pressure of mechanical abrasion on these hemangiectic lamellae caused their rupture and hemorrhage (Fig. 8). Fish exposed to 0.37 mg/l CPO had high numbers of hemangiectic lamellae.

Discussion

The spot, *Leiostomus xanthurus*, showed responses to lethal and sublethal concentrations of CPO similar to those reported for freshwater, estuarine and marine teleosts. Concentrations of 0.13 to 0.37 mg/l CPO were acutely toxic to spot after brief exposure (88 to 400 minutes) but lower concentrations of 0.09 and 0.12 mg/l did not result in mortalities during 2,880 minutes of exposure.

A very narrow range of concentrations of chlorination, eliciting rapid toxicological or physiological responses or no effect, have also been observed by other investigators. Rainbow trout, *Salmo gairdneri*, exposed to sequential 0.5 mg/l chlorination pulses in flowing water tests showed extremely irregular breathing; the observed irregularity was much less pronounced in fish exposed to 0.4 mg/l. On the basis of irregular breathing patterns Bass and Heath (1977) suggested that an important response threshold for rainbow trout exists between 0.4 and 0.5 mg/l chlorination. Brook trout, *Salvelinus fontinalis* (Mitchell), also tested in flowing water, showed marked changes in response when exposed to a small range of chlorination concentrations (Dandy 1972). At 0.35 and 0.08 mg/l chlorination an increase in activity, ventilation and "coughing" reflex was noted. However, at 0.04 and 0.005 mg/l chlorination, the initial increases were less pronounced and lasted only about 2 hours. Dandy (1972) therefore concluded that a threshold (for modified activity) occurred between 0.08 mg/l, a concentration which resulted in acute toxicity after 18 hours and 0.04 mg/l where fish lived for 48 hours. At the lowest exposure concentration of 0.005 mg/l chlorination, fish were still alive after 7 days of exposure even though they elicited a general decline in activity.

Middaugh, et al. (1977b) observed an acute toxicological response in spot (LT50 < 1,000 minutes) at exposure concentrations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ranging from 0.08 to 2.48 mg/l chlorination, but no deaths after exposure to 0.02 and 0.04 mg/l for 8 days. Spot exposed to 0 22 to 2.48 mg/l chlorination showed a rapid increase in ventilation rate and activity, those exposed to 0.08 and 0.18 mg/l usually demonstrated a gradual increase in ventilation rate and accumulation of copious quantities of mucus along the posterior edge of the operculum. While no obvious changes in ventilation rates or behavior were observed for the 0.02 and 0.04 mg/l chlorination concentrations, an apparent decline in feeding response (compared to controls) occurred during the 8 day exposure at these concentrations.

In this study, the change in opercular ventilation rates seems to be related to the exposure concentrations of CPO and the resultant lethal or sublethal response. At each of the lethal concentrations, spot showed a rapid increase in opercular ventilation rates. These increases were generally 2 to 3 times those measured for control fish. Additionally, ventilation rates decreased just prior to death.

For sublethal concentrations of CPO, smaller increases in opercular ventilation rates were observed in comparison to controls. These increases were short lived with ventilation rates returning to values only slightly higher than observed for controls by 720 minutes after beginning the exposure to a sublethal concentration of 0.09 mg/l CPO and by 1,440 minutes at a sublethal concentration of 0.12 mg/l CPO.

An increase in opercular ventilation rates in spot (Table 2) during exposure to CPO is probably linked to the development of low blood P_{O_2}. Copious amounts of mucus were noted in fish exposed to lethal chlorination levels; and in conjunction with the observed damage to gill epithelial tissues, may have served to partially block the transport of gases at the gill, thus resulting in depressed rates of oxygen uptake (Table 4).

Blood pH responses for spot compare favorably with those observed in other investigations of the toxicity of chlorination to fish. Rainbow trout, *Salmo gairdneri*, showed decreases in blood pH following exposure to a 0.5 mg/l spike pulse of chlorination (total duration of pulse 3 hours). Subsequent pulse exposure (3 times daily at 0700, 1500 and 2300 hours) resulted in further decreases in pH with little recovery between pulses (Bass and Heath 1977). In white perch, *Morone americana*, an estuarine fish, Block, et al. (1978) measured a blood pH of 7.5 for controls. After 7 hours of exposure to a measured concentration of 0.8 mg/l CPO, the blood pH had decreased to 6.8.

Spot fish hemoglobin possesses a Root effect (Bonaventura, et al. 1976) which shifts the oxygen equilibrium curve down and to the right with decreasing pH. The decreases in blood pH in spot thus lead to a reduction in the ability of the blood to transport oxygen, causing even lower oxygen uptake rates. The reductions in oxygen uptake rates are greater with increasing CPO concentrations (and thus greater changes in blood pH). The highest CPO concentration 0.37 mg/l shows a very low $\dot{V}_{O_2}$ (119.33 $ml \cdot kg \cdot hour^{-1}$) and probably reflects the extensive structural damage of the gas exchange surfaces (Figs. 7 and 8; Middaugh,

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Fig. 1. *Leiostomus xanthurus*. Normal branchium and secondary lamellae of control fish; note slight focal artifactual separation of respiratory epithelium from underlying tissues. ×300.

Fig. 2. Sloughing of respiratory epithelium in fish exposed to a concentration of 0.09 mg/l CPO. ×300.

Fig. 3. Sloughing and fusion of respiratory epithelium from lamellae of fish exposed to a concentration of 0.13 mg/l CPO. ×300.

Fig. 4. Sloughing and fusion of respiratory epithelium in fish exposed to a concentration of 0.13 mg/l CPO; note some fluid in space between respiratory epithelium and circulatory tissue. ×667.

Fig. 5. Denudation of circulatory tissues in lamellae of fish exposed to a concentration of 0.20 mg/l CPO. ×300.

Fig. 6. Complete denudation of circulatory tissues in fish exposed to 0.37 mg/l CPO; note that remnants of respiratory epithelia are removed to ends of secondary lamellae. ×300.

Fig. 7. Hemangiectic secondary lamella in fish exposed to a concentration of 0.13 mg/l CPO. ×300.

Fig. 8. Rupture and hemorrhage of hemangiectic lamella in fish exposed to a concentration of 0.13 mg/l CPO. ×300.

et al. 1977a, b), and reduced blood O_2 content resulting from decreased blood pH.

Reductions in $\dot{V}_{O_2}$ at sublethal CPO concentrations may be related to mucus covering the gill epithelial tissues as well as to the structural damage observed (Figs. 2 and 3). At both sublethal CPO concentrations opercular ventilation rates initially increased but returned to control levels after 1,440 minutes (24 hours). Although the elevated opercular ventilation rates are not as high as those found in the lethal CPO concentrations, they may be indicative of low blood P_{O_2}. The subsequent decrease in ventilation rates to near control values and the depressed rates of oxygen uptake may reflect a type of acclimation to an obstruction to gas exchange.

Our % methemoglobin measurements are in general agreement with those of Block, et al. (1978) who found only slight and statistically insignificant increases in the % methemoglobin in white perch, *Morone americana*, exposed to chlorination. In contrast, Buckley, et al. (1976), Bass and Heath (1977) and Zeitoun (1977) all observed small but statistically significant increases in the % methemoglobin for Coho salmon, *Oncorhynchus kisutch*, or rainbow trout, *Salmo gairdneri*, exposed to chlorination. Buckley, et al. (1976) and Bass and Heath (1977) questioned the biological significance of the increased % methemoglobin which they measured, while Zeitoun (1977) suggests that the effects of increased % methemoglobin in combination with hemolytic anemia are biologically significant. In the present study, extremely low levels of methemoglobin in both control and experimental animals (Table 3) suggests that this factor is not important in inducing mortality in spot during CPO exposure.

Gill tissue damage in the present study was observed for CPO concentrations ranging from 0.09 to 0.37 mg/l. Similar gill tissue alteration in chlorination exposed fish has been reported by Bass, et al. (1977) for the blue gill, *Lepomis marochirus*, and rainbow trout, *Salmo gairdneri*; Middaugh, et al. (1977a, b) for striped bass, *Morone saxatilis*, and spot, *Leiostomus xanthurus*; Cohen and Valenzuela (1977) for the mosquitofish, *Gambusia affinis*; and Block, et al. (1978) for the white perch, *Morone americana*.

Most damage found in gills of spot during this study parallels and is similar to that found by other authors who have examined chlorination exposed fish. The finding of hemangiectic lamellae (Figs. 7, 8), however, is similar to findings made by Smart (1976) in rainbow trout exposed to various ammonia concentrations, and has not been previously reported for fish exposed to chlorination. The hemorrhagic results of any pressure applied to the hemangiectic lamellae would cause increased debilitation and weakness concurrent with respiratory and osmoregulatory stress caused by severe denudation of gill primary structures in chlorine-exposed fish.

In summary, the primary site of toxic action in CPO exposed spot appears to be the gill tissue epithelium. Observed increases in opercular ventilation rates, decreases in blood pH, slight elevation in the % methemoglobin and decreased whole animal $\dot{V}_{O_2}$ probably are secondary or tertiary effects resulting from respiratory and perhaps osmoregulatory dysfunction.

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